Heterocyclic Amines: Occurrence and Prevention in Cooked Food

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This article deals with the mutagenic heterocyclic amines, especially the aminoimidazoazaarenes family, isolated from cooked foods. The conditions which lead to their occurrence in foods are discussed. This formation primarily depends on the characteristics of the food, such as the type of the food and the presence of precursors, water, and lipids. Secondarily, it depends on the cooking modes where the temperature is considered to be the most important factor involved in their formation. As their formation during cooking represents a health risk, we present some ways and means to limit their formation by alternative cooking methods that tend to decrease heterocyclic amine concentrations in foods as they are implicated in cancer risks. *Key words*: aminoimidazoazaarenes, cooking methods, health risk, heterocyclic amines, mutagenicity, prevention. *Environ Health Perspect* 104:280–288 (1996)

Food processing and other culinary preparations enable food products to be made edible, increase their appetizing appeal, and provide stability during storage. However, certain food processing procedures could have a negative impact on consumer health. This is the case for fried and grilled food. Ikeda et al. (*1*) have shown that populations that frequently consume grilled fish, or even charred fish, have a high incidence of gastric cancers.

Since the 1960s, genetically toxic substances have been isolated from processed foods. Since then, many studies have identified these compounds and tested their genetic toxicity and carcinogenicity in animals in order to estimate the risks associated with these substances. The main compounds concerned are the nitrosamines, the polycyclic aromatic hydrocarbons (PAHs), the heterocyclic amines (HCAs), and certain products of the Maillard reaction.

The presence of heterocyclic amines has been shown by Sugimura and coworkers in 1977 (*2*). These authors determined that the mutagenic activity of grilled beef or fish as well as the activity found in smoke condensates is far greater than the activity attributed solely to the PAHs present in these food. The level of exposure to these HCAs seems to be about the same as that of the nitrosamines or benzo[*a*]pyrene (*3*).

Once metabolically activated, certain heterocyclic amines are among some of the most powerful mutagenic agents that have been detected up until now by the Ames test [*Salmonella typhimurium* TA 98, (*4*)]. They demonstrate mutagenic activity towards mammalian cells in culture and may cause chromosome aberrations in mouse cells (*5,6*). The first metabolic step in the hepatic activation is a microsomal oxidation of the exocyclic amino group, which is primarily catalyzed by cytochromes P450IA1 and especially P450IA2 (*7*). The *N*-hydroxyamino derivatives are further esterified to form more reactive species (*8*).

In humans, the initial activation step is thought to be N-oxidation by CYP1A2 (cytochrome P4501A2) (*9*). The *N*-hydroxy arylamine metabolite is *O*-acetylated in the liver or transported to the appropriate target organ where it is *O*-acetylated by the polymorphic *N*-acetyltransferase (NAT2) to form an arylamine-DNA adduct (*10*).

Heterocyclic amines have been shown capable of inducing organ tumors in mice, rats and monkeys (*11–16*). In addition, their effects seem synergic (*17,18*). The sensitivity of the animal depends on the sex of the animal, females being more receptive than the males (*19*).

There are two major classes of genetically toxic heterocyclic amines present in foods: aminoimidazoazaarenes and carbolines.

The aminoimidazoazaarene (AIAs) compounds have a 2-aminoimidazo group fused to a quinoline (IQ and MeIQ), a quinoxaline (MeIQx, and DiMeIQx), or a pyridine (PhIP) ring (*20*).

The carbolines and its analogues of the type "non-IQ," contrary to the AIAs (type "IQ"), include the aminopyridoindoles (Trp-P-1, Trp-P-2, AαC, MeαC), the aminopyridoimidazoles (Glu-P-1, Glu-P-2, Lys-P-1, Orn-P-1), and an aminophenylpyridine (Phe-P-1) (*21*). In addition, genetically toxic heterocyclic amines are present in cooked grain products, but, as yet, have not been clearly identified (*22–24*).

This article discusses the conditions which lead to the formation of genetically toxic heterocyclic amines in food the ways in which their presence in food may be reduced, and why it is desirable to do so.

Genotoxic Heterocyclic Amines in Cooked Foods

Measuring the amount of HCAs in food is both difficult and delicate. Up until 1990, it was not possible to simultaneously quantify the different compounds present. This explains why most of the previous work studying the formation and the presence of these substances in foods has been conducted in an indirect manner, by measuring the amount of bacterial mutagenicity in so-called basic fractions (that is, fractions of samples containing HCAs). The species of bacteria used are relatively specific to HCAs, but the mutagenicity may be modified by other food constituents present within the test fractions (*25*). In an attempt to overcome this problem, some workers separate out the different components of the basic fractions using chromatography (high performance liquid chromatography, HPLC), before carrying out the test. In this way, comparable chromatographic profiles may be established. The respective roles played by the HCAs of the type IQ and those of the type non-IQ in the overall mutagenicity observed may be determined by treating the fractions with nitrites in an acid medium. This selective treatment inactivates the compounds of the type non-IQ (*26*).

The results obtained so far by studying mutagenicity may not be considered as definitive, especially as the most abundant amines found in terms of mass such as PhIP and 4'-OH PhIP in fried beef (*27*), show weak mutagenic activity in bacteria (*4*). The studies conducted using the Ames test are resumed here when no other equivalent study with identification of amines is available. However, quantitative assays on specific amines, and more recently on the totality of these genetically toxic substances found within specific foodstuffs have not, up until now, shown to be contradictory to previous results obtained by the observation of mutagenicity. HCAs are generated during heat treatment. The characteristics of the food and the type of heat treatment used determine their formation (*28*).

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Characteristics of the Food

Type of Food

The basic fractions obtained from protein rich foods show a higher level of mutagenicity than those fractions obtained from carbohydrate rich foods that have been submitted to the same heat treatments (*22,29*). Among protein rich foods, the formation of basic mutagenic compounds during heating varies greatly. The greatest mutagenic activity is seen in meat (beef, pork, lamb, and chicken), fish (*30–34*), beef extracts and the residues obtained after cooking meat (*35,36*), and beef flavors (*37*). Meat juices formed during cooking meat may contain an equivalent amount of mutagenic activity to that found in fried meat (*36,38*). A significant level of mutagenic activity is also found in commercial sauces and meat stocks. The mutagenic behavior seen here is probably due to the beef extract used for their preparation.

On the other hand, no such activity is seen in stock cubes prepared from vegetable produce (*35*) and no HCAs were detected in processed flavoring based on hydrolyzed plant proteins (*39*). Basic extracts of meat and fish that have undergone the same heat treatment show the same mutagenic profiles (*38,40–42*). They therefore contain the same mutagenic compounds (*43–55*), as shown in Table 1.

Milk, cheese, eggs, beans, some types of peas, and offal are only susceptible after severe treatment. The appearance of mutagenic activity is accompanied by a change of color or charring (*56*). For instance, in standard heat treatments of milk (pasteurization, sterilization, ultra high temperature, and spray dehydration) there is no mutagenic agent formation in the basic fractions (*57*). Only certain types of egg preparations exhibit mutagenic activity. For example, fried eggs with signs of charring or egg yolk oil that has been slightly charred, as in the preparation of ranyu, a Japanese dietetic food, show a high level of mutagenic activity (*58,59*).

The research conducted on carbohydrate-rich foods seems contradictory. According to Taylor and coworkers (*60*), deep fry chips do not show mutagenic activity unless they become burned and are no longer edible. On the other hand, Spingarn et al. (*29*) have shown that potatoes fried in a frying pan under domestic conditions, toast, or oven cooked biscuits showed a significant level of mutagenic activity, albeit, very inferior to the activity observed in meat (*61*). However these mutagenic agents have not been clearly identified and their specificity towards the species of bacteria used is not as great as the specificity of mutagenic agents produced in meat. Hageman et al. (*62*), suggest that the mutagenic activity of deep fry chips is not due to the HCAs known, but due to other compounds. This has also been suggested recently by Knize and coworkers (*23,24*) concerning the mutagenic activity of foods derived from grain products, especially those with high gluten content. Gluten has been found to be quite mutagenic when heated (*22*). Preliminary data suggest that the mutagens may be HCAs, but they appear distinct in structure from the 18 previously described mutagens derived from cooked muscle meat products.

The Precursors of AIAs

The main precursors of the AIAs found in meat and fish are creatine/creatinine, free amino acids and sugars (*63–65*). The discovery that reducing sugars, amino acids, and creatine are AIA precursors led to speculation that the Maillard reaction plays an important role in the formation of these mutagens. Jagerstad et al. (*63,65*) have suggested that the amino-imidazo part of AIA compounds arises from creatine by cyclization to creatinine with water elimination. Strecker degradation products, such as pyridines or pyrazines formed in the Maillard reaction between hexose and amino acids, are believed to form the remainder of the molecule. Aldol condensation is thought to link the two parts together by means of a Strecker aldehyde. Another possible origin of AIA compounds is an initial aldol condensation of creatinine with formaldehyde or acetaldehyde, followed by the conjugation of pyridine or pyrazine (*67*). Lee et al. (*68*) have reported evidence for AIA formation in a model system of 2-methylpyridine, acetylformaldehyde, and creatinine.

Creatine. It is well understood that the basis for the mutagenic activity of muscle meats is the presence of creatine stored in high concentration in these tissues (*69*). During heating, creatine is transformed into creatinine from which the imidazole part of AIA is formed (*70,71*). Higher processing temperatures lead to a more rapid decrease in creatine and increase in creatinine. Most of this conversion seems to occur in the first 40 min of processing (*72*). No correlation has been found between creatine or creatinine concentrations in different meats and formation of mutagenic activity (*73*).

It is therefore an essential precursor. There are many arguments to back up this hypothesis: adding creatine or creatinine to meat or fish during heat treatment leads to a net increase in the formation of mutagenic agents of the IQ type (*64,74,75*); protein-rich foods containing little or no creatine (cheese, tofu, beans, liver, kidney, shrimp, and plants) produce very few mutagenic agents (*33,76*); models systems containing creatine or creatinine generate AIAs (*71,77*); creatine has been demonstrated to form a part of the PhIP molecule by using radiolabeled creatine (*74*); and beef flavors with high creatine (1.5 mg/g) or creatinine (2 mg/g) levels exhibited higher mutagenic activities than did flavor with low levels of these compounds (*37*).

However, the amount of mutagenic agents formed during heat treatment are not always strictly related to the amount of creatine/creatinine present. This is why bonito or cooked tuna fish that have equivalent amounts of creatine/creatinine to certain other fish show higher levels of mutagenic agents of the IQ type. Other precursors probably exist (e.g., guanidine) (*78*).

Amino acids. Studies carried out on model systems show that amino acids or short chain peptides are absolutely necessary for the formation of AIAs (*75*). On the contrary to the addition of creatine/creatinine, the mutagenic profiles may change depending on the type of amino acid added. Also, the same mutagenic molecule may be produced by different amino acids: threonine, glycine, lysine, alanine and serine all lead to the formation, often simultaneously, of MeIQx and DiMeIQx (*77*).

The amounts of mutagenic agent formed varies depending on the type of

Table 1. Heterocyclic amine concentrations in cooked meat/fish

Food type	Cooking method	Mutagens (ng/g)[a] PhIP	AαC	IQ	DiMeIQx	MeIQx	Temperature (°C)[b]	References
Beef, ground	Fried	0–21.5	—	0–1.8	0–9.35	0–8	150–230	(*43*)
Beef steak	Broiled/fried	0.6–48.5	1.2–89	0.19	0.1–1.3	0.5–5,1	150–225	(*3,44,45*)
Beef extract	Boiled	0–3.62	0	0–8	0–28	0–42.4		(*45–49*)
Chicken	Broiled	38.1	180	—	0.81	2.33		(*3,47,50*)
Lamb	Broiled	42.5	2.5	—	0.67	1.01		(*3,47*)
Pork	Fried	1.5–36	—	0.01–0.04	0.24–9.3	0.4–26.7	155–180	(*43,51,52*)
Fish	Fried/broiled/ barbecued	1.7–73	0–109	0.16–20	0.1	0–6.44	200–270	(*51,53–55*)

[a]Mutagen concentrations represent the optimal and minimal values reported.
[b]Temperatures are reported only when they were indicated by the authors.

amino acid: threonine, glycine, lysine, and serine are among the precursors that result in the greatest amounts of mutagenic agents obtained in model systems (amino acid + sugar + creatine) (*75*).

When amino acids are added to meat extracts or minced meat before cooking, the results obtained are contradictory. Some studies have shown that certain amino acids lead to an increased yield of mutagenic agents (*79,80*) whereas Ashoor et al. (*81*) only obtained an increased mutagenicity with proline, that is, one out of 17 amino acids tested. There again, Jones and Weisburger (*82*) have shown that the addition of tryptophan or proline before cooking leads to an inhibition in the formation of mutagenic agents. According to these authors, this inhibition could be due to the aldehydes precursors of mutagenic agents that react preferentially with the nitrogen of the pyrrole group of these two amino acids rather than with the creatinine. Differences in the experimental conditions (type of meat, time and temperature, and cooking time) may be the reason for these observed differences.

The amino acids, indispensable precursors, seem to act in a qualitative manner on the formation of AIAs, but, on the quantitative side other factors seem to be more important. No AIAs are formed from amino acids incorporated in proteins (*75*).

Sugars. The presence of sugars is not strictly necessary for the formation of mutagenic agents (*21*). AIAs have been obtained in the absence of sugars or aldehydes in model systems using dry heat treatments. However, in liquid model systems their presence at particular concentrations is necessary. The maximum production of mutagenic agents is obtained using a level which is equivalent to half that of creatine or amino acids (*20,83*). On the other hand, when the quantity of sugar present is equal to or greater than that of these two reactants, the formation of mutagenic agents is greatly reduced: this has been shown for glucose (*83*), fructose, sucrose, and lactose (*20*). These data from liquid model systems confirm the work carried out on meat (*75*).

In addition, the amount of mutagenic agents produced depends on the sugar or the aldehyde used in these model systems. Thus, an optimal production of PhIP has been obtained using erthyrose and glyceraldehyde (*84*). Glucose has been shown to be incorporated in different HCAs (*85*). Fructose and ribose are more efficient than glucose (*86*). Sucrose and lactose also generate AIAs (*20*). Free sugars are probably not the only possible precursors. Under certain conditions, nucleic acids may induce the formation of PhIP (*87*).

Role of Water and Lipids

As we have already seen, under certain conditions the HCAs may form in the total absence of water (*79,88,89*). During cooking, water soluble mutagenic agent precursors, migrate with the water towards the surface of the food where they are then exposed to the temperature required for the reactions leading to the formation of HCAs (*79,90*). The external surfaces of grilled meat show greater levels of mutagenic activity than the inner part where normally the center temperature is lower (*91*). The mutagenicity of foods may be greatly reduced by preventing the evaporation of water during cooking (*33*). Above an initial optimal level of water in the food, such as 40% for beef (*92*), mutagenic activity is also reduced, probably because of a dilution effect on the precursors.

The role played by intrinsic lipids in the development of mutagenic activity is not clear. However, they do seem to play an important role in the formation of HCAs. Most authors agree that there exists an optimum level of intrinsic lipids necessary for a maximum formation of mutagenic agents for Spingarn et al. (*93*). In the case of minced meat, this level is 10%. Not long after this, Barnes and Weisburger (*94*) found that in beef patties containing 11% or 25% of lipids after cooking, the amount of IQ produced is 4 times greater for the higher level of lipids. In the case of grilled beef, at 180° or 240°C, a maximum mutagenicity was seen when the level of lipids reached 15% (*95,96*). The differences found for the optimum level of lipids is probably related to the differences in the experimental procedures used. Most studies investigating the influence of lipids on the formation of mutagens in meat systems have used the Ames test. The effects of fats might have been underestimated, since the Ames test is inhibited by long-chain fatty acids (*94*).

A number of hypotheses have been put forward in order to explain the role of lipids: some researchers believe that increasing the level of lipids up to the optimal value may favor the transfer of heat (*97*). Above this value, the reduction in the formation of mutagenic agents could be due either to a reduction in cooking time (*98*), or a dilution effect on the precursors (*96,97*). The addition of lipids to model systems increases the mutagenic activity and the yield of HCAs (*99–101*). For example, corn oil or olive oil added to model system containing creatine, glycine, and glucose dissolved in water, heated at 180°C for 30 min, almost doubled the yield of MeIQx compared to the yield without fat (*101*).

Modifying Factors of Mutagenic Activity and HCA Formation

A number of other food constituents are involved in the formation of HCAs.

Antioxidants, ascorbic acid (*102,103*), butylhydroxyanisol (BHA) (*104*), and certain concentrations of mixtures of tocopherols (*76*) show an inhibitory action, likewise for the inhibitors of nonenzymic browning. Sodium bisulfite for example, totally inhibits the formation of HCAs in canned foods when added at the level of 0.5% (102). Butylhydroxytoluene (BHT), on the other hand, promotes the formation of HCAs of the quinoxaline type (*105*).

In beef grilled in the presence of soya protein, chlorogenic acid, or cotton grain flour, a reduced mutagenic activity is seen (*104,106*). The inhibitory effect of the soya proteins might be related to the presence of phenolic compounds (*107*), or to its water binding capacity, leading to a higher water content in the cooked product with less transport of the precursors to the surface (*28*).

Certain phenolic compounds more or less complex (tannic acid, quercetol, rutin, catechin, and propyl gallate) seem to reduce the amount of AαC formed during albumine pyrolysis (*108*). Recently, Weisburger and coworkers (*109*) showed that tea polyphenols may represent another approach to lower HCA formation.

In addition, flavones reduce the formation of mutagenic agents. This property may be explained by the reduced formation of certain products of the Maillard reaction that are precursors of HA formation (*106*). Flavones or flavonols that contain C5, C7 and C4′ hydroxyl groups are potent inhibitors of P-450 enzyme activities induced by Aroclor 1254 (P450IA1 and P450IA2), and may potentially be useful as chemopreventive agents against HCA-induced mutagenesis or carcinogenesis (*110*). Other products of the Maillard reaction are inhibitors of HCA formation (*111*). The possible mechanisms of the antimutagenic effect of Maillard reaction products prepared from xylose and lysine to IQ has been suggested to be due to the interaction of this Maillard product with proximate metabolites of IQ to form inactive adducts and not to inhibit the activity of hepatic microsomal enzymes, direct reaction with intact IQ or interaction with DNA (*112*). The presence of iron increases the formation of mutagenic agents, and this increase is opposed by the addition of a chelating agent, EDTA (*100*).

Cooking Modes

Effects of the Temperature and Cooking Time

The reactions leading to the formation of AIAs follow the classic laws of chemistry; that is, the formation of the mutagenic agents increases with the temperature and cooking time (*31,32,113,114*). Temperature is the most important of the two factors involved in formation of mutagenic agents. Most of the mutagenic activity measured by Ames test, can be accounted for by the known HCAs present in fried beef meat (*43*).

The onset of mutagenicity in meat and meat extracts was found at temperature of 100°C (*41,91*). The authors did not identify the mutagenic compounds. The mutagenic compounds began to form in meats and model systems at temperatures of 150°C or higher (*20,70,72,115*) and the concentrations of the AIAs increased with processing temperature (*20,43,72,113*).

At all processing temperatures, there was a time lag before AIAs could be detected (*31,72,116*). This lag could be related to the time required for the mixture or meat surface to reach 100°–150°C.

Mutagenic activity or AIA formation in muscle products or in model systems increased with processing time at 150°–175°C. However, at higher temperature (190°–250°C), the concentrations of HCAs increased during an initial time of processing and then decreased or plateaued (*7,20,43,53*).

The presence of carbolines in food is mainly observed after heat treatment involving high temperatures. Carbolines were not commonly found in the Western diet (*21*). But recently, Layton and coworkers (*117*) reported the dietary intake of a carboline (AαC) in commonly consumed foods as greater than MeIQx, DiMeIQx, and IQ. On the other hand, in this work, we address only the genotoxic compounds, but nongenotoxic compound production (harman, non-harman) is possible as well (*118*) and these substances can act as co-mutagens (*119*).

Types of Cooking and the Preparation of the Food

Generally speaking, the types of cooking that involve temperatures of around 100°C (boiling in water, steaming, stewing without previous browning, poaching, or braising) lead to a production of mutagenic agents that is too low to be quantifiable (*92*). Microwave ovens (not using a grill that may be added) do not lead to the formation of these mutagenic agents (*41,120–123*). No significant nutritional differences exist between foods prepared by conventional and microwave methods (*124*).

Microwave pretreatment has been proposed as a practical way to reduce fat and HCA content of fried ground beef (*125*). However, additional studies determining HCA reduction in other meat products and possible changes in taste and texture of the meat, need to be explored. In a recent work, Jonker and Til (*126*) reported that rodent diets cooked by microwave compared with those cooked conventionally resulted in no toxicity.

Cooking methods such as roasting and baking, which heat food by indirect convection, produce low or intermediate levels of mutagenic activity in most protein-rich foods (*34,56,92,117*). Cooking procedures that heat foods by radiative and conductive processes (grilling, frying) lead to an increased mutagenic activity (*92*). Reheating or keeping food warm does not alter the amount of mutagenic agents present (*122*). On the other hand, pretreatments such as freezing or steaming in preserved foods may lead to an increased formation during further preparations. These preliminary treatments bring about the destruction of cell walls, which could lead to the liberation of precursors (*127*).

The use of thickeners for sauces has little effect on the mutagenicity produced (*36*). High levels of mutagenic agents formed are found in the crust, cooking juices, residues left in the frying pan and the vapors given off in cooking (*36,79*).

Using fats (butter, margarine, or oils) in cooking greatly increases in the amounts of mutagenic agents formed when the temperatures are high (more than 200°C) (*41,128,129*).

The type of surface used for cooking (steel, aluminum, cast iron, teflon, enamel or ceramic) does not lead to any difference in the amount of mutagenic agents formed when the meat is subjected to the same length of cooking time (*130*).

Discussion

The concentrations of HCAs in cooked foods reported on Table 1 show that HCA formation during the cooking of food may represent a health risk. However, in our opinion, a real average of each HCA is difficult to determine because of incomplete information of their food concentrations. Generally, measurements concern only some of the HCAs and cooking conditions are not always clearly defined. Moreover, HCA studies had been done in specific countries and would probably not reflect worldwide cooking modes. In France, such studies are lacking.

To determine the human risk related to daily intake of HCAs, the same study should be conducted at the same time in several laboratories and in different countries, especially since food and drink is not the only source of exposure. Air, rain water, fire smoke, exhaust fumes, and cigarette smoke (*131*) as well as indoor pollution related to cooking and other preparation processes contain HCAs (*132–134*).

The western diet is considered to contain the highest levels of AIAs (*135*). This is probably due to the abundance of meat in the diet that is cooked at temperatures above 200°C. However, the problem is worldwide: many studies carried out in a number of countries show that industrial or traditional (household) cooking lead to the generation of HCAs (*37,54,123,136–138*).

Today, the relationship between diet and cancer is well established. Certain epidemiological studies have shown a high fecal mutagenicity in populations with a high risk of colorectal cancers (*139,140*). The ingestion of fried foods (even with little or no fat added) has also been associated with other cancers such as pancreatic cancer (*141*) or urothelial cancer (*142*). The authors suggest that this association could be due the high level of formation of mutagenic agents during cooking. A close relationship between colorectal cancer and eating red meat as main food has been found (*143*) and the incidence of cancer could be closely related to cooking modes (*144,145*). Eating well-done meat leads to a risk 3.5 times greater that seen on consuming rare meat (*146*). Also, a high level of risk is associated with the consumption of cooking juices from meat (*141*).

In parallel to epidemiological studies, experimental studies on animals have shown that even at small doses, the HCAs can induce the formation of adducts in the DNA within different organs such as the liver, the kidneys, the colon, and the stomach (*146*). Recently, Snyderwine et al. (*147*) showed a wide PhIP–DNA adduct distribution with an accumulation in certain monkey tissues.

The authors concluded that the presence of such adducts in nontarget organ (heart, aorta) might have toxicological consequences.

In rodents and monkeys, the HCAs have been shown to be carcinogenic (*12, 148,149*). In rats and mice, there are multiple target organs of HCAs. All compounds, except for PhIP (*148,150*) proved to be carcinogenic to the liver. PhIP, which is mostly excreted via the feces (*151*), induces a high incidence of cancers of the colon and of the mammary glands in the rat (*14,148*).

In the case of MeIQx, the earlier in life that it is administered, the greater the DNA adduct formation (*152*). When MeIQx or PhIP were administrated to neonatal mice, they induced tumors with doses lower by 5000–10000 times than those that are effective in adults (*153*). Recently, a transplacental transfer of PhIP in pregnant rodents and from lactating animals to suckling pups (*154,155*) was reported. Furthermore, presence of DNA adducts in the tissues of pups suggest that this route of exposure may have a carcinogenic consequence to the newborn. PhIP from breast milk lactating rats undergoes metabolic activation in 5-day-old rat pups (*156*). These findings may have carcinogenic and toxicological implications for the offspring who breast-feed and consume a diet rich in cooked meat. According to Felton (*157*), it appears appropriate to examine human breast milk for HCAs and their metabolites.

It seems likely that human populations that consume large quantities of meat also ingest appreciable quantities of carcinogens of the HA type produced during the cooking. It is possible that the correlation between meat consumption and the incidence of colorectal cancers, mostly related to fats and animal proteins (*158*), could at least partially be due to the ingestion of HCAs.

Modern knowledge in this field seems to culminate in the same line of arguments, but, no definite proof is available. Also, the metabolic capacities of HCAs differ from subject to subject and certain populations could cause groups to be at risk (*159*).

It is certain that the carcinogenic effect of these substances is modified (positively or negatively) by other substances present in food, but, more detailed studies need to be conducted.

It therefore seems reasonable to limit the formation of these HCAs to which we are all exposed by frying, grilling, meat juices, residues, and cooking fumes. It is important to evacuate these cooking fumes, especially from restaurant and canteen kitchens in order to avoid the continuous exposure of the staff. The total elimination of exposure to HCAs from food sources is not realistic, but a reduction is possible. The easiest way to do this is to reduce the cooking temperature (100°–130°C). Ways to do this could be by consistently using cooking techniques such as water baths, cooking in water, steam cooking, loosely covering, or using microwave ovens.

Protein-rich foods of a muscular origin present high risks due to their composition and the typical ways in which they are prepared (grilling, frying, and roasting at high temperatures which leads to a high water loss). For these products, the modes of cooking previously cited are not always appreciated as they do not allow the same aromas to develop. In this case it would be preferential to adapt the traditional ways of cooking in order to limit the formation of HCAs. Although the mutagenic activity may be markedly decreased by frying at lower temperatures, there is obviously no pan temperature at which mutagenic activity is not formed (*28*).

For oven cooking, using a fan-heated oven or an oven with a covered heating element help avoid the formation of carcinogens related to grease spilling from the utensil onto the heat source. It is also possible to cover food in grease-proof paper. Precooking beef meat in a microwave has been suggested (*90,123*), draining away any meat juices formed (rich in precursors) before frying or traditional cooking. However, additional studies determining HA reduction in other meat products and possible changes in taste, texture, and nutritional content of the meat still need to be explored.

When using a grill or barbecue, the food must be placed far enough from the heat source to avoid any contact with the flames. Using grills where the heat source is vertical is a good idea in order to avoid drops of fat dripping onto the wood or charcoal. These droplets, when exposed to high temperatures, are at the origin of fumes rich in carcinogens that eventually fall back onto the food. Generally speaking, eating charred food as well as using meat juices or residues for preparing sauces should be avoided. Cooking utensils should be covered to avoid water evaporation. Also, for industrial preparations of beef extracts or products based on meat, the different stages of concentration by evaporation must be closely controlled or eventually reconsidered.

These preventive methods concerning the formation of HCAs should also allow the limitation of the appearance of other newly formed, unwanted substances, (such as polycyclic aromatic hydrocarbons; (PAHs). However, the formation of HCAs often goes hand in hand with the formation of organoleptic qualities that are sought after. Strict prevention is possible if the consumer modifies not only his habits but also his tastes. But this does not correspond to contemporary tendencies, as shown by a recent American survey (*160*).

The use of inhibitors has been studied. For example, the incorporation of tryptophan or proline in a sauce for minced beef has been described by Jones and Weisburger (*82*). In our opinion, if this method allows AIA reduction, it would not prevent carboline formation (e.g., Trp-P1, Trp-P2) during overheating that may occur during house cooking because tryptophan is carboline precursor. In addition, AαC, MeIQx could be generated in high amounts.

The addition of glucose to meat before cooking is another possibility to limit mutagen formation. Adding small amounts of glucose favors the production of mutagenic agents, but in excess it plays a role of inhibitor (*161*). The fact that sugars in excess are able to limit the formation of mutagens could be exploited by the industrial field in certain meat products where carbohydrates are added to the recipe. However, studies are still needed to evaluate mutagen formation in meat products containing ingredients rich in starch and carbohydrates.

Using soya proteins and antioxidants (*104,105*) and the addition of flavones (*162*) are possible mutagen formation limiters. Cooking vegetables or fruits rich in flavones in conjunction with the meat allows a reduction in the mutagenicity. *In vitro*, the presence of an antimutagenic activities against HCAs in many fruits and vegetables has been demonstrated (*163*).

The intake of dietary fiber seems to be protective of colon cancer (*164*). A fiber supplementation in the form of whole wheat and oat fiber has been shown to decrease the mutagenicity in the feces of healthy volunteers (*165*). A binding capacity for some type of dietary fibers to mutagenic pyrolysates both *in vitro* and *in vivo*, has been observed (*166–169*). Recently, wheat bran has been shown to bind to hydrophobic mutagen (MeIQx) in the diet and this binding can be enhanced after fermentation under colonic conditions (*170*).

Individuals who consume a typical diet with high meat and fat content have an elevated risk of colon cancer. There is however, epidemiological evidence that the consumption of cereals, fiber, and vegetables, especially cruciferous vegetables, has a protective effect against this cancer (*171*). A peroxidase activity, *in vitro*, has been found to be present in broccoli, cauliflower, green beans, and tomatoes which may contribute to the antimutagenic activities in these vegetables (*163*). These considerations, however, are speculative and should be regarded with great caution since knowledge about the identity of antimutagenic factors and reaction mechanisms is limited and there is a real need for *in vivo* investigations.

Another dietary factor that has been implicated *in vitro* and *in vivo* as having an effect of mutagenesis and carcinogenesis is the intake of milk products, in particular

fermented milk products. An antimutagenic effect of *Lactobacillus acidophilus* has been observed in the total fecal and urinary mutagen excretion of healthy subjects who consumed fried beef patties supplemented with *L. acidophilus* fermented milk (*172*). Recently, it has been shown that IQ compounds can be bound to bacteria from the normal intestinal microflora *in vitro* (*173*). These results can give rise to speculations regarding the value and the possibility of increasing the number of bacterial cells in the intestinal tracts of humans consuming fried food, by administration of bacterial cells. According to the authors, the intake of lactic acid producing strains appears to be the most appropriate.

In addition, another path to explore is the production of grilled or fried flavoring that would not contain AIAs. These may then be added to food that has been cooked at reduced temperatures.

To conclude, the contemporary risks linked to the ingestion of heterocyclic amines are not clearly defined, but all the authors agree that it is important to reduce the amounts of mutagenic agents and carcinogens formed during cooking by modifying the conditions used for heating. We do not think for our own sake, that it is reasonable to wait until the proof is available, as this could take a long time. The ideas provided in this article are easy to carry out, but, the ideas have to be implemented by consumers. This implies the collaboration of the medical core (doctors, nutritionists, and dieticians) in order to inform the consumers. At the time of submission of this manuscript, a paper has been published (*174*) which reports HCA formation in chicken using particular cooking methods, especially for PhIP at substantially higher levels than has been reported previously in red meat. Although the link between consumption of HCAs and excess cancer risk in humans has yet to be demonstrated, this paper reinforces our conclusions concerning necessary HCA prevention.

References

1. Ikeda M, Yoshimoto K, Yoshimura T, Kono S, Kato H, Kurastune M. A cohort study on the possible association between broiled fish intake and cancer. Gann 74:640–648 (1983).
2. Sugimura T, Nagao M, Kawachi T, Honds M, Yahagi T, Seino Y, Sato S, Matsukura N, Matsushima T, Shirai A, Sawamura M, Matsumoto H. Mutagens-carcinogens in foods, with special reference to highly mutagenic pyrolytic products in broiled foods. In: Origins of human cancer (Hiatt HH, Watson JD, Winsten JA, eds). New York:Cold Spring Harbor Laboratory, 1977;1561–1577.
3. Wakabayachi K, Ushiyama H, Takayashi M, Nukaya H, Kim SB, Hirose M, Ochiai M, Sugimura T, Nagao M. Exposure to heterocyclic amines. Environ Health Perspect 99:129–133 (1993).
4. Brams A, De Meester C. Mutagenic potency of heterocyclic amines towards Salmonella *Typhimurium*. Possible causes of variability in the results observed. Mutat Res 280:103–107 (1992).
5. Tucker JD, Carrano AV, Allen NA, Christensen ML, Knize MG, Stout CL, Felton JS. In vivo cytogenetic effects of cooked food mutagens. Mutat Res 224:105–113 (1989).
6. Aeschbacher HV, Turesky RJ. Mammalian cell mutagenicity and metabolism of heterocyclic aromatic amines. Mutat Res 259:235–250 (1991).
7. Gonzales FJ, Gelboin HV. In: New Horizons in Biological Dosimetry: Human cytochromes P450: evolution, catalytic activities and interindividual variations in expression (Gledhill BF, Mauro F, eds). New York:Wiley-Liss, 1991;11–20.
8. De Flora S, Bennicelli C, D'Agostini F, Izzotti A, Camoirano AA. Cytosolic activation of aromatic and heterocyclic amines. Inhibition by dicoumarol and enhancement in viral hepatitis B. Environ Health Perspect 102:69–74 (1994).
9. Turesky RJ, Lang NP, Butler MA, Teilel CH, Kadlubar FF. Metabolic activation of carcinogenic heterocyclic amines by human liver and colon. Carcinogenesis 12:1839–1845 (1991).
10. Sinha R, Rothman N, Brown ED, Mark SD, Hoover RN, Caporaso NE, Levander OA, Knize MG, Lang NP, Kadlubar FF. Pan-fried meat containing high levels of heterocyclic aromatic amines but low levels of polycyclic aromatic hydrocarbons induces cytochrome P4501A2 activity in humans. Cancer Res 54:6154–6159 (1994).
11. Adamsson RH. Mutagens and carcinogens formed during cooking of foods and methods used to minimize their formation. Cancer Prev 1:1–7 (1990).
12. Ohgaki H, Takayama S, Sugimura T. Carcinogenicities of heterocyclic amines in cooked food. Mutat Res 259:399–410 (1991).
13. Bogen KT. Cancer potencies of heterocyclic amines found in cooked foods. Food Chem Toxicol 32:505–515 (1994).
14. Ghoshal A, Preisegger KH, Takayama S, Thogeirsson SS, Snyderwine EG. Induction of mammary tumors in female Sprague-Dawley rats by the food-derived carcinogen 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine and effect of dietary fat. Carcinogenesis 15:2429–2433 (1994).
15. Nagao M, Sugimura T. Carcinogenic factors in food with relevance to colon cancer development. Mutat Res 290:43–51 (1993).
16. Thorgeirsson UP, Dalgard DW, Reeves J, Adamson RH. Tumor incidence in a chemical carcinogenesis study of nonhuman primates. Regul Toxicol Pharmacol 19:130–151 (1994).
17. Takayama S, Nakatsuru Y, Sato S. Carcinogenic effect of the simultaneous administration of five heterocyclic amines to F344 rats. Gann 78:1068–1072 (1987).
18. Hasegawa R, Tanaka H, Tamano S, Shirai T, Nagao M, Sugimura T, Ito N. Synergistic enhancement of small and large intestinal carcinogenesis by combined treatment of rats with five heterocyclic amines in a medium-term multi-organ bioassay. Carcinogenesis 15:2567–2573 (1994).
19. Matsukura N, Kawachi T, Morino K, Ohgaki H, Sugimura T. Carcinogenicity in mice of mutagenic compounds from a tryptophan pyrolysate. Science 213:346–347 (1981).
20. Skog K, Jägerstad M. Effects of monosaccharides and disaccharides on the formation of food mutagens in model systems. Mutat Res 230:263–272 (1990).
21. Felton JS, Knize MG. Heterocyclic-amine mutagens/carcinogens in foods. In: Chemical carcinogenesis and mutagenesis (Cooper CS, Grover PL, ed). New York:Springer Verlag, 1990;471–502.
22. Friedman M, Wilson RE, Ziderman II. Mutagen formation in heated wheat gluten, carbohydrates, and gluten/carbohydrate blends. J Agric Food Chem 38:1019–1028 (1990).
23. Knize MG, Cunningham PL, Griffin EA, Jones AL, Felton JS. Characterization of mutagenic activity in cooked-grain-food products. J Agric Food Chem 32:15–21 (1993).
24. Knize MG, Cunningham PL, Avila AL, Nes AL, Griffin EA, Felton JS. Formation of mutagenic activity from amino acids heated at cooking temperatures. J Agric Food Chem 32:55–60 (1993).
25. Ames BN, McCan J, Yamasaki E. Methods for detecting carcinogens and mutagens with *Salmonella*/mammalian microsome mutagenicity test. Mutat Res 31:347–361 (1975).
26. Tsuda M, Negishi C, Makino R, Sato S, Yamaizumi Z, Hirayama T, Sugimura T. Uses of nitrite and hypochlorite treatments in determination of the contribution of IQ-type and nonIQ-type heterocyclic amines to the mutagenicities in crude pyrolyzed materials. Mutat Res 147:335–341 (1985).
27. Felton JS, Knize MC, Shen NH, Levis PR, Andersen BD, Happe J, Hatch FT. The isolation and identification of a new mutagen from fried ground beef: 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP). Carcinogenesis 7:1081–1086 (1986).
28. Skog K. Cooking procedures and food mutagens: a literature review. Food Chem Toxicol 31:655–675 (1993).
29. Spingarn NE, Slocum LA, Weisburger JH. Formation of mutagens in cooked foods. II. Foods with high starch content. Cancer Lett 9:7–12 (1980).
30. Commoner B, Vithayathil AJ, Dolara P, Nair S, Madyastha P, Cuca GC. Formation of mutagens in beef and beef extract during cooking. Science 20:913–916 (1978).
31. Spingarn NE, Weisburger JH. Formation of mutagens in cooked food. I. Beef. Cancer Lett 7:259–264 (1979).
32. Overvick E, Nilsson L, Fredholm L, Levin O, Nord CE, Gustafsson JA. High mutagenic activity formed in pan-broiled pork. Mutat Res 135:149–157 (1984).
33. Kikugawa K, Kato T. Formation of mutagen 2-amino-3,8-dimethylimidazo(4,5-f) quinoxaline (MeIQx) and 2-amino-3,4,8-trimethylimidazo (4,5-f) quinoxaline(4,8- DiMeIQx), in heated fish meats. Mutat Res 179:5–14 (1987).
34. Doolittle DJ, Rahn CA, Burger CT, Lee C K, Reed B, Riccio E. Effect of cooking methods on the mutagenicity of food and on urinary mutagenicity of human consumers. Food

Chem Toxicol 27:657–666 (1989).
35. Dolara P, Bianchini F. Genotoxicity studies of cooked food. In: Nutritional and toxicological aspects of food processing (Walker R, Quattrucci E, eds), New York:Taylor Francis, 1988;125–137.
36. Overvick E, Nilsson L, Fredholm L, Levin O, Nord C E, Gustafsson JA. Mutagenicity of pan residues and gravy from fried meat. Mutat Res 187:47–55 (1987).
37. Jackson LS, Hargraves WA, Stoup WH, Diachenko GW. Heterocyclic aromatic amine content of selected beef flavors. Mutat Res 320:113–124 (1994).
38. Knize MG, Shen NH, Felton JS. The production of mutagens in cooked foods (paper 88-130.5). In: Proceedings of Air Pollution Control Association, 81st Annual Meeting, 19–24 June 1988, Dallas, Texas;1–18.
39. Gross GA, Grüter A, Heyland S. Optimization of the sensitivity of high-performance liquid chromatography in the detection of heterocyclic aromatic amine mutagens. Food Chem Toxicol 30:491–498 (1992).
40. Nielsen PA, Vahl M, Gry J. HPLC profiles of mutagens in lean ground pork fried at different temperatures. Zeischrift-fûr Lebensmittel-Untersuchung und-Forschung, 187:451–456 (1988).
41. Barrington PJ, Baker RSU, Truswell AS, Bonin AM, Ryan AJ, Paulin AP. Mutagenicity of basic fractions derived from lamb and beef cooked by common household methods. Food Chem Toxicol 28:141–146 (1990).
42. Felton JS, Knize MG. Occurrence, identification and bacterial mutagenicity of heterocyclic amines in cooked food. Mutat Res 259: 205–217 (1991).
43. Knize MG, Dolbeare FA, Carroll DL, Moore DH, Felton JS. Effect of cooking time and temperature on the heterocyclic amine content of fried beef patties. Food Chem Toxic 32:595–603 (1995).
44. Murray S, Lynch AM, Knize MG, Gooderham NJ. Quantification of the carcinogen 2-amino-3,8-dimethyl- and 3,4,8-trimethyl[4,5-f]quinoxaline and 2-amino-1-methylimidazo[4,5-b] pyridine in food using a combined assay based on capillary column gas chromatography-negative ion mass spectrometry. J Chromatogr 616:211–219 (1993).
45. Gross GA. Simple methods for quantifying mutagenic heterocyclic aromatic amines in food products. Carcinogenesis 11:1597–1603 (1990).
46. Gross GA, Philippossian G, Aeschbacher HU. An efficient and convenient method for the purification of mutagenic heterocyclic amines in heated meat products. Carcinogenesis 10:1175–1182 (1989).
47. Hayatsu H, Arimoto S, Wakabayashi K. Methods for separation and detection of heterocyclic amines. In Mutagens in food, detection and Prevention (Hayatsu H, ed). Boca Raton, FL:CRC Press, 1991;101–112.
48. Takahashi M, Wakabayashi K, Nagao M, Yamamoto M, Masui T, Goto T, Kinae N, Tomita I, Sugimura T. Quantification of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) in beef extract by liquid chromatography with electrochemical detection (LCEC). Carcinogenesis 6: 1195–1199 (1985).
49. Hargraves WA, Pariza MW. Purification and mass spectral characterization of bacterial mutagens from commercial beef extract. Cancer Res 43:1467–1472 (1983).
50. Masumoto T, Yoshida D, Tomita H. Determination of mutagens, amino-alpha-carbolines in grilled foods and cigarette smoke condensate. Cancer Lett 12:105–110 (1981).
51. Dragsted LO. Exposure and carcinogenicity of heterocyclic amines. In: Proceedings of the Toxicology Forum, 1992 annual European meeting, 1–5 June, Copenhagen, Denmark, 1992;141–148.
52. Vahl M, Gry J, Nielsen PA. Mutagens in fried pork and the influence of the frying temperature. Proceedings of the XVII Annual Meeting of the European Environmental Mutagen Society, Zurich 99 (1987).
53. Gross GA, Grüter A. Quantification of mutagenic carcinogenic heterocyclic aromatic amines in food products. J Chromatogr 592:271–278 (1992).
54. Zhang XM, Wakabayashi K, Liu ZC, Sugimura T, Nagao M. Mutagenic and carcinogenic heterocyclic amines in chinese cooked foods. Mutat Res 201:181–188 (1988).
55. Kasai H, Yamaizumi Z, Nishimura S, Wakabayashi K, Nagao M, Sugimura T, Spingarn NE, Weisburger JH, Yokoyama S, Miyazawa T. A potent mutagen in broiled fish. Part 1. 2-amino-3-methyl-3H-imidazo[4,5-f]quinoline. J Chem Soc 1:2290–2293 (1981).
56. Bjeldanes LF, Morris MM, Felton JS, Healy S, Stuemer D, Berry P, Timourian H, Hatch FT. Mutagens from the cooking of food. III. Survey by Ames Salmonella test of mutagen formation in secondary sources of cooked dietary protein. Food Chem Toxicol 20:365–369 (1982).
57. Berg HE, Van Boekel MAJS, Jongen WMF. Heating milk: a study on mutagenicity. J Food Sci 55:1000–1003 (1990).
58. Grose KR, Grant JL, Bjeldanes JF, Andresen BD, Healy SK, Felton JS, Hatch FT. Isolation of the carcinogen IQ from fried egg patties. J Agric Food Chem 34:201–202 (1986).
59. Kato T, Kikugawa K, Asanoma M, Sakabe Y. Occurrence of 2-amino-3-methylimidazo [4, 5-f] quinoline (IQ),2-amino-6-methyldipyrido [1,2-a:3',2'-d] imidazole (Glu-P-1) and other heterocyclic amine mutagens in oil of charred egg yolk (ranyu). Mutat Res 240:259–266 (1990).
60. Taylor SL, Berg CM, Shoptaugh NH, Traisman E. Mutagen formation in deep-fat fried foods as a function of frying conditions. J Assoc Off Anal Chem 60:576–580 (1983).
61. Spingarn N, Kasai H, Vuolo L, Nishimura S, Yamaizumi Z, Sugimura T, Matsushima T, Weisburger JH. Formation of mutagens in cooked foods. III. Isolation of a potent mutagen from beef. Cancer Lett 9:177–183 (1980).
62. Hageman G, Hermans R, TenHoor F, Kleinjans J. Mutagenicity of deep-frying fat and evaluation of urine mutagenicity after consumption of fried potatoes. Food Chem Toxicol 28:75–80 (1990).
63. Jägerstad M, Laser-Reutersward A, Olson R, Grivas S, Nyhammar T, Olson K, Dahlquist A. Creatin(ine) and Maillard reaction products as precursors of mutagenic compounds: effects of various amino acids. Food Chem 12:255–264 (1983).
64. Knize MG, Shen NH, Felton JS. A comparison of mutagen production in fried ground chicken and beef: effect of supplemental creatin. Mutagenesis 3:503–508 (1988).
65. Felton JS, Knize MG. Occurrence, identification and bacterial mutagenicity of heterocyclic amines in cooked foods. Mutat Res 259:205–217 (1991).
66. Jägerstad M, Skog K, Grivas S, Olsson K, Laser Reuterswärd A, Negishi C, Sato S. Formation of food mutagens via Maillard reactions. In: Genetic toxicology of the diet, (Knudsen I, ed), New York:Wiley-Liss, 1986;155–167.
67. Nyhammar T. Studies on the Maillard reaction and its role in the formation of food mutagens (PhD dissertation). Uppsala: Swedish University of Agricultural Sciences, 1986.
68. Lee H, Lin MY, Hao NJ. Effects of Maillard reaction products on mutagen formation in boiled pork juice. Mutagenesis 10:179–183 (1995).
69. Jägerstad M, Grivas S, Olsson K. Formation of heterocyclic amines using model systems. Mutat Res 259:219–233 (1991).
70. Laser Reuterswärd A, Skog K, Jagerstad M. Effects of creatine and creatinine content on the mutagenic activity of meat extracts, bouillons and gravies from different sources. Food Chem Toxicol 25:747–754 (1987).
71. Laser-Reutersward A, Skog K, Jagerstad M. Mutagenicity of pan-fried bovine tissues in relation to their content of creatine, creatinine, monosaccharides and free amino-acids. Food Chem Toxicol 25:755–762 (1987).
72. Jackson LS, Hargraves WA. Effects of time and temperature on the formation if MeIQx and DiMeIQx in a model system containing threonine, glucose, and creatine. J Agri Food Chem 43:1678–1684 (1995).
73. Viske R, Joner PE. Mutagenicity, creatine and nutrient contents of pan fried meat from various animal species. Acta Vet Scand 34:1–7 (1993).
74. Felton JS, Knize MG. Mutagen formation in muscle meats and model heating systems. In: Mutagens in food: detection and prevention (Hayatsu H, ed). Boca Raton, FL:CRC Press, 1991;58–66
75. Jägerstad M, Laser Reuterswärd R, Öste A, Dahlquist A, Grivas S, Olsson K, Nyhammar T. Creatinine and Maillard reaction products as precursors of mutagenic compounds formed in fried beef. In: The Maillard reaction in foods and nutrition. ACS Symposium series 215 (Waller GR, Feather MS, eds). Washington, DC:American Chemical Society 1983;507–519.
76. Chen C, Pearson AM, Gray JI. Meat mutagens. Adv Food Nutr Res 34:387–449 (1990).
77. Jägerstad M, Skog K. Formation of meat mutagens. In: Nutritional and toxicological consequences of food processing (Friedman M, ed). New York:Plenum Press, 1991; 83–105.
78. Marsh NL, Iwaoka WT, Mower HF. Formation of mutagens during the frying of Hawaiian fish: correlation with creatine and creatinine content. Mutat Res 242:181–186 (1990).
79. Overvick E, Kleman M, Berg I, Gustafsson JA. Influence of creatine, amino acids and water on the formation of the mutagenic heterocyclic amines found in cooked meat. Carcinogenesis 10:2293–2301 (1989).

80. Taylor RT, Fultz E, Knize MG. Mutagen formation in a model beef boiling system. III. Purification and identification of three heterocyclic amine mutagens-carcinogens. J Environ Sci Health 20:135–148 (1985).
81. Ashoor S, Dietrich R, Chu F, Pariza M. Proline enhances mutagen formation in ground beef during frying. Life Sci 26:1801–1805 (1980).
82. Jones C, Weisburger J. L-tryptophan inhibits formation of mutagens during cooking of meat and in laboratory models. Mutat Res 206:343–349 (1988).
83. Skog K, Jägerstad M. Effects of glucose on the formation of PhIP in a model system. Carcinogenesis 12:2297–2300 (1991).
84. Manabe S, Kurihara N, Wada O, Tohyama K, Aramaki T. Formation of PhIP in a mixture of creatinine, phenylalanine and sugar or aldehyde by aqueous heating. Carcinogenesis 13:827–830 (1992).
85. Skog K, Jagerstad M. Incorporation of carbon atoms from glucose into the food mutagens MeIQx and 4,8-DiMeIQx using ^{14}C-labelled glucose in model system. Carcinogenesis 10:2027–2031 (1993).
86. Matsushima T, Muramatsu M. Formation of MeIQx and 4,8-DiMeIQX by heating mixtures of creatinine, amino-acids and monosaccharides. In: Genetic toxicology of the diet (Knudsen I, Alan R, eds), New York:Wiley-Liss, 1986;330.
87. Manabe E, Kurihara N, Shibutani T, Wada O, Ueki A, Suzuki H. Nucleic acids induce the formation of a carcinogen, 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) in a model system. Carcinogenesis 14:903–906 (1993).
88. Taylor RT, Fultz E, Knize MG. Mutagen formation in a model beef supernatant fraction. IV. Properties of the system. Environ Health Perspect 67:59–74 (1986).
89. Yoshida D, Fukuhara Y. Formation of mutagens by heating creatine and amino acids. Agric Biol Chem 46:1069–1070 (1982).
90. Felton JS, Knize MG, Dolbeare FA, Wu R. Mutagenic activity of heterocyclic amines in cooked foods. Environ Health Perspect 102:201–204 (1994).
91. Dolara P, Commoner B, Vithayathil A, Cuca G, Tuley E, Madyastha P, Nair S, Kriebel D. The effect of temperature on the formation of mutagens in heated beef stock and cooked ground beef. Mutat Res 60:231–237 (1979).
92. Bjeldanes LF, Morris MM, Felton JS, Healy S, Stuermer D, Berry P, Timourian H, Hatch S. Mutagens from the cooking of food. II. Survey by Ames/Salmonella test of mutagen formation in the major protein-rich foods of the american diet. Food Chem Toxicol 20:357–363 (1982).
93. Spingarn N, Garvie-Gould C, Vuolo L, Weisburger JH. Formation of mutagens in fried beef patties. Cancer Lett 13:93–97 (1981).
94. Barnes WS, Weisburger JH. Lipid content and mutagen formation in the cooking of beef. Proc Am Assoc Cancer Res 24:65 (1983).
95. Felton JS, Knize MG, Healy SK, Shen NH, Lewis P, Hatch FT, Bjeldanes LF. Characterization and identification of the mutagens in cooked beef: effect of fat content and cooking conditions. Environ Mutagen 6:437 (1984).
96. Knize MG, Andresen BD, Healy SK, Shen NH, Lewis P, Bjeldanes LF, Hatch FT, Felton JS. Effect of temperature, patty thickness and fat content on the production of mutagens in fried ground beef. Food Chem Toxicol 23:1035–1040 (1985).
97. Chen C. Factors influencing mutagen formation during frying of ground beef (PhD dissertation). East Lansing, MI:Michigan State University, 1988.
98. Holtz E, Skjöldebrand C, Jägerstad M, Laser-Reuterswärd A, Isberg PE. Effects of recipes on crust formation and mutagenicity in meat products during baking. J Food Technol 20:57–66 (1985).
99. Barnes WS, Maher JC, Weisburger JH. High pressure liquid chromatographic method for the analysis of 2-amino-3methyl[4,5-f]imidazo quinoline, a mutagen formed from the cooking of food. J Agric Food Chem 31:883–886 (1983).
100. Barnes WS, Weisburger JH. Formation and inhibition of mutagens during frying of beef and relationship to fat content. Proc Am Assoc Cancer Res 25:102 (1984).
101. Johansson M, Skog K, Jägerstad M. Effects of edible oils and fatty acids on the formation of mutagenic heterocyclic amines in a model system. Carcinogenesis 14:89–94 (1993).
102. Krone CA, Yeh SMJ, Iwaoka WT. Mutagen formation during commercial processing of foods. Environ Health Perspect 67:75–88 (1986).
103. Pearson AM, Chen C, Grey JI. Effects of different antioxidants on formation of meat mutagens during frying of ground beef. Proceeding of 38th International Congress of Meat Science Technology, 23–28 August 1992, Clermont-Ferrand, France; 567–570.
104. Wang YY, Vuolo LL, Spingarn NE, Weisburger JH. Formation of mutagens in cooked foods. V. The mutagen reducing effect of soy protein concentrates and antioxidants during frying of beef. Cancer Lett 16:179–189 (1982).
105. Pearson AM, Chen C, Ian Gray J, Aust SD. Mechanisms involved in meat mutagen formation and inhibition. Free Radical Biol Med 13:161–167 (1992).
106. Lee H, Jiaan CY, Tsai SJ. Flavon inhibits mutagen formation during heating in a glycin/creatine/glucose model system. Food Chem 45:235–238 (1992).
107. Rhee KS, Donelly KC, Ziprin YA. Reduction of mutagen formation in fried ground beef by glandless cottonseed flour. J Food Prot 50:753–755 (1987).
108. Fukuhara Y, Yoshida D, Goto F. Reduction of mutagenic products in the presence of polyphenols during pyrolysis of protein. Agric Biol Chem 45:1061–1066 (1981).
109. Weisburger JH, Nagao M, Wakabayashi K, Oguri A. Prevention of heterocyclic amine formation by tea and tea polyphenols. Cancer Lett 83:1–2 (1994).
110. Lee H, Wang HW, Su HY, Hao NJ. The structure-activity relationships of flavonoids as inhibitors of cytochrome P-450 enzymes in rat liver microsomes and the mutagenicity of 2-amino-3-methyl-imidazo[4,5-f] quinoline. Mutagenesis 9:101–106 (1994).
111. Yen GC, Chan CF. Inhibition by xylose-lysine Maillard reaction products of the formation of MeIQx in a heated creatinine, glycine and glucose model system. Biosci Biotechnol Biochem 57:664–665 (1993).
112. Yen GC, Hsieh PP. Possible mechanisms of antimutagenic effect of Maillard reaction products prepared from xylose and lysine. J Agric Food Chem 42:133–137 (1994).
113. Bjeldanes LF, Morris MM, Timourian H, Hatch FT. Effects of meat composition and cooking conditions on mutagenicity of fried ground beef. J Agric Food Chem 31:18–21 (1983).
114. Hatch FT, Felton JS, Bjeldanes LF. Mutagens from the cooking of food: thermic mutagens in beef. CRC. Crit Rev Toxicol 1:147–163 (1982).
115. Rappaport SM, McCartney MS, Wei ET. Volatilization of mutagens from beef during cooking. Cancer Lett 8:139–145 (1979).
116. Pariza MW, Ashoor SH, Chu FS. Effects of temperature and time on mutagen formation in pan-fried hamburger. Cancer Lett 7:63–69 (1979).
117. Layton DW, Bogen KT, Knize MG, Hatch FT, Johnsson VM, Felton JS. Cancer risk of heterocyclic amines in cooked foods:an analysis and implications for research. Carcinogenesis 16:39–52 (1995).
118. Kleinbauer S, Rabache M. The carboline formation by tryptophan or protein-heat treatments. Sci des Aliments 10:417–428 (1990).
119. Nagao M, Yhagi T, Kawachi T, Seino Y, Honda M, Matsukura N, Sugimira T, Wakabayashi K, Tsuji K, Kosuge T. Mutagens in foods and especially pyrolysis products of protein. In: Progress in genetic toxicology (Scott D, Bridges BA, Sobels FH, eds). Elseiver, 1977;259–264.
120. Nader CJ, Spencer LK, Weller RA. Mutagen production during pan-broiling compared with microwave irradiation of beef. Cancer Lett 13:147–151 (1981).
121. Baker R, Arlauskas A, Bonin A, Angus D. Detection of mutagenic activity in human urine following fried pork or bacon meals. Cancer Lett 16:81–89 (1982).
122. Berg I, Overvick E, Gustafsson JA. Effect of cooking time on mutagen formation in smoke, crust and pan residue from pan-broiled pork. Food Chem Toxicol 28:421–426 (1990).
123. Felton JS, Knize MG, Roper M, Fultz E, Shen NH, Turteltaub KW. Chemical analysis, prevention, and low-level dosimetry of heterocyclic amines from cooked food. Cancer Res (Suppl) 52:2103s–2107s (1992).
124. Cross GA, Fung DYC. The effect of microwaves on nutrient value of foods. Crit Rev Food Sci Nutr 16:355–381 (1982).
125. Felton JS, Fultz E, Dolbeare FA, Knize MG. Effect of microwave pretreatment on heterocyclic aromatic amine, mutagens/carcinogens in fried beef patties. Food Chem Toxicol 32:897–903 (1994).
126. Jonker D, Til HP. Human diets cooked by microwave or conventionally: comparative sub-chronic (13-wk) toxicity study in rats. Food Chem Toxicol 33:245–256 (1995).
127. Krone CA, Iwaoka WT. Mutagen formation in processed foods. In: Xenobiotics in feeds and foods (Finley JW, Schwass DC, eds), ACS Symposium 234, Washington, DC, 1983;117–127.
128. Nilsson L, Overvick E, Fredholm L, Levin O, Nord CE, Gustafsson JA. Influence of frying fat on mutagenic activity in lean pork meat. Mutat Res 171:115–121 (1986).
129. Berg I, Overvick E, Nord CE, Gustafsson JA. Mutagenicity in smoke formed during broiling

of lean pork at 200, 250 and 300°C. Mutat Res 207:199–204 (1988).
130. Bjeldanes LF, Felton JS, Hatch FT. Mutagens in cooked food. ACS Symp Ser 234:149–168 (1983).
131. Manabe S, Kurihara N, Wada O, Izumikawa S, Asakuno K, Morita M. Detection of a carcinogen, 2-amino-1-methyl-6-phenylimidazo [4, 5-b] pyridine, in airborne particles and diesel-exhaust particles. Environ Pollution 80:281–286 (1993).
132. Löfroth G. Airborne mutagens and carcinogens from cooking and other food preparation processes. Toxicol Lett 72:83–86 (1994).
133. Thiebaud HP, Knize MG, Kuzmicky PA, Felton JS, Hsieh DP. Mutagenicity and chemical analysis of fumes from cooking meat. J Agric Food Chem 42:1502–1510 (1994).
134. Nardini B, Granella M, Clonfero E. Mutagens in indoor air particulate. Mutat Res 322:193–202 (1994).
135. Felton JS, Knize MG, Shen NH, Andresen BD, Bjeldanes LF, Hatch FT. Identification of the mutagens in cooked beef. Environ Health Perspect 67:17–24 (1986).
136. Tikkanen LM. Sources of mutagenicity in cooked finnish foods. Food Chem Toxicol 29:87–92 (1991).
137. Alink GM, Knize MG, Shen NH, Hesse SP, Felton JS. Mutagenicity of food pellets from human diets in the Netherlands. Mutat Res 206:387–393 (1988).
138. Starvic B, Matula TI, Klassen R, Downiet RH. Analysis of commercial bouillons for trace levels of mutagens. Food Chem Toxicol 31:981–987 (1993).
139. Schiffman MH. Epidemiology of fecal mutagenicity. Epidemiol Rev 8:92–105 (1986).
140. Schiffman MH, Van Tassel RL, Robinson A. A case-control study of colorectal cancer and fecapentaene excretion. Cancer Res 49:3420–3424 (1989).
141. Norell SE, Ahlbom A, Erwald R, Jacobson G, Lindberg-Navier I, Olin R, Törnberg B, Wiechel KL. Diet and pancreatic cancer: a case-control study. Am J Epidemiol 131:376–378 (1986).
142. Steineck G, Hagman U, Gerhardsson M, Norell SE. Vitamin A supplements, fried foods, fat and urothelial cancer. A case-referent study in Stockholm in 1985–1987. Int J Cancer 45:1006–1011 (1990).
143. Willet WC, Stampfer MJ, Colditz GA, Rosner BA, Speizer FE. Relation of meat, fat and fiber intake to the risk of colon cancer in a prospective study among women. New Engl J Med 323:1664–1672 (1990).
144. Schiffman MH, Felton JS. Fried foods and the risk of colon cancer. Letter to the Editor. Am J Epidemiol 131:376–378 (1990).
145. Gerhardsson De Verdier M, Hagman U, Peters RK, Steineck G, Overvick E. Meat, cooking methods and colorectal cancer: a case-referent study in Stockholm. Int J Cancer 49:520–525 (1991).
146. Snyderwine EG, Yamashita K, Adamson RH. Use of the 32P-postlabeling method to detect DNA adducts of 2-amino-3-methyl-imidazo (4,5-f) quinoline (IQ) in monkeys fed IQ: identification of the N-(deoxyguanosin-8-YL)-IQ adduct. Carcinogenesis 9:1739–1743 (1988).
147. Snyderwine EG, Schut HAJ, Sugimura T, Nagao M, Adamsson RH. DNA adduct levels of PhIP in tissues of cynomolgus monkeys after single or multiple dosing. Carcinogenesis 15:2757–2761 (1995).
148. Ito N, Hasegawa R, Sano M, Tamano S, Esumi H, Takayama S, Sugimura T. A new colon and mammary carcinogen in cooked food, 2-amino-1-methyl-6-phenylimidazo(4,5-b) pyridine (PhIP). Carcinogenesis 12:1503–1506 (1991).
149. Adamsson RH, Thorgeirsson UP, Snyderwine EG, Thorgeirsson SS, Reeves J, Dalgard DW, Takayama S, Sugimura T. Carcinogenicity of IQ in nonhuman primates:induction of tumors in three macaques. Jpn J Cancer Res 81:10–14 (1990).
150. Esumi H, Ohgaki H, Kohsen E, Takayama S, Sugimura T. Induction of lymphoma in CDF_1 mice by the food mutagen PhIP. Jpn J Cancer Res 80:1176–1178 (1989).
151. Watkins BE, Esumi H, Wakabayashi K, Nagao M, Sugimura T. Fate and distribution of 2-amino-1-methyl-6-phenylimidazo (4,5-b) pyridine (PhIP) in rats. Carcinogenesis 12:1073–1078 (1991).
152. Yamashita K, Adachi M, Kato S, Nakayama H, Ochiai M, Wakabashi K, Sato S, Nagao M, Sugimura T. DNA adducts formed by 2-amino-3,8-dimethylimidazo[4,5-f] quinoline in rat liver: dose-response on chronic administration. Jpn J Cancer Res 81:470–476 (1990).
153. Dooley KL, Von Tungeln LS, Bucci T, Fu PP, Kadlubar FF. Comparative carcinogenicity of 4-aminobiphenyl and the food pyrolysates, Glu-P-1, IQ, PhIP, and MeIQx in the neonatal B6C3F1male mice. Cancer Lett 62:205–209 (1992).
154. Ghoshal A, Snyderwine EG. Excretion of food-derived heterocyclic amine carcinogens into breast milk of lactating rats and formation of DNA adducts in the newborn. Carcinogenesis 14:2199–2203 (1993).
155. Brittebo EB, Karlsson AA, Skog KI, Jägerstad IM. Transfer of the food mutagen PhIP to foetuses and newborn mice following maternal exposure. Food Chem Toxicol 32:717–726 (1994).
156. Davis CD, Ghoshal A, Scut HAJ, Snyderwine, EG. Metabolism of the food-derived carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine by lactating 344 rats and their nursing pups. J Natl Cancer Inst 86:1065–1070 (1994).
157. Felton JS. A carcinogenic heterocyclic amine, common in food, and its metabolites are found in rodent breast milk and urine of the suckling pups. J Natl Cancer Inst 86:1041–1042 (1994).
158. Amstrong B, Doll R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. Int J Cancer 15:617–631 (1975).
159. Hein DW, Doll MA, Rustan TD, Gray K, Feng Y, Ferguson RJ, Grant DM. Metabolic activation and deactivation of arylamine carcinogens by recombinant human NAT1 and polymorphic NAT2 acetyltransferases. Carcinogenesis 14:1633–1638 (1993).
160. McIntosh WA, Christensen LB, Acuff GR. Perceptions of risks of eating undercooked meat and willingness to change cooking practices. Appetite 22:83–96 (1994).
161. Skog K, Jagerstad M, Laser Reuterswärd A. Inhibition effects of carbohydrates on the formation of food mutagens in fried beef patties. Food Chem Toxicol 30:681–688 (1992).
162. Hertog MGL, Hollman PC, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. Nutr Cancer 20:21–29 (1993).
163. Edenharder R, Kurz P, John K, Burgard S, Seeger K. *In vitro* effect of vegetable and fruit juices on the mutagenicity of 2-amino-3-methylimidazo [4,5-f] quinoline, 2-amino-3,4-dimethylimidazo [4,5-f] quinoline and 2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline. Food Chem Toxicol 32:443–459 (1994).
164. Willett W. The search for the causes of breast and colon cancer. Nature 338:389–393 (1989).
165. Reddy BS, Sharma C, Simi B, Engle A, Laakso K, Puska P, Korpela R. Metabolic epidemiology of colon cancer: effect of dietary fiber on fecal mutagens and bile acids in healthy subjects. Cancer Res 47:644–648 (1987).
166. Lindeskog P, Övervik E, Nilsson L, Nord CE, Gustafsson JA. Influence of fried meat and fiber on cytochrome P-450 mediated activity and excretion of mutagens in rats. Mutat Res 204:553–563 (1988).
167. Sjödin P, Nyman M, Nilsson N, Asp G, Pulusani S. Effect of feeding fermented milk on the incidence of ^{14}C-labeled food mutagens (IQ, MeIQ, MeIQx) by dietary fiber in vitro. J Fd Sci 50:1680–1684 (1985).
168. Vikse R, Mjelva BB, Klungsoyr L. Reversible binding of the cooked food mutagen MeIQx to lignin enriched preparation from wheat bran. Food Chem Toxicol 30:239–246 (1992).
169. Ferguson LR, Roberton AM, Watson ME, Kestell P, Harris PJ. The adsorption of a range of dietary carcinogens by cellulose, a model insoluble dietary fiber. Mutat Res 319: 257–266 (1993).
170. Ryder R, Robertson JA. The effect of fibre source and fermentation on the apparent hydrophobic binding properties of wheat bran preparations for the mutagen MeIQx. Carcinogenesis 16:209–216 (1995).
171. Greenwald P. Colon cancer overview. Cancer 70:1206–1215 (1992).
172. Lidbeck A, Övervick E, Rafter J, Nord CE, Gustafsson JA. Effect of *Lactobacillus acidophilus* supplements on mutagen excretion in faeces and urine in humans. Microb Ecol Health Dis 5:59–67 (1992).
173. Orrhage K, Sillerstom E, Gustafsson JA, Nord CE, Rafter J. Binding of mutagenic heterocyclic amines by intestinal and lactic bacteria. Mutat Res 311:239–248 (1994).
174. Sinha R, Rothman N, Brown ED, Salmon C, Kinze M, Swanson A, Rossi S, Mark S, Levander O, Felton J. High concentration of the carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) occur in chicken but are dependent on the cooking method. Cancer Res 55:516–4519 (1995).